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Single-chain variable fragment intrabody impairs LPS-induced inflammatory responses by interfering with the interaction between the WASP N-terminal domain and Btk in macrophages

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ABSTRACT

Wiskott–Aldrich syndrome protein (WASP) plays important roles in both acquired and innate immune responses. We recently uncovered that the WASP N-terminal domain specifically binds the Src homology (SH) 3 domain of Bruton's tyrosine kinase (Btk) in macrophages. Over-expression of the WASP N-terminal domain impairs LPS-induced inflammatory responses. To evaluate the significance of this interaction in LPS signaling, we established bone marrow-derived macrophage (BMDM) cell lines from transgenic (Tg) mice expressing anti-WASP N-terminal domain single-chain variable fragment (scFv) intrabody. Anti-WASP scFv specifically bound endogenous WASP and inhibited its specific binding to the SH3 domain of Btk in the Tg BMDMs. Tyrosine phosphorylation in WASP was inhibited after LPS stimulation. As a result, TNF-α, IL-6, and IL-1β gene transcription and NF-κB phosphorylation were impaired. These observations strongly suggest that the phosphorylation of WASP by Btk plays a pivotal role in transducing the LPS signaling pathway in macrophages.

1. Introduction

Wiskott–Aldrich syndrome (WAS) is an X-linked immunodeficiency caused by various types of genetic mutations in WAS protein (WASP) [1]. WASP is mainly expressed in hematopoietic cells, and symptoms of WAS are consistent with cytoskeletal aberrations in these cells. WASP plays important roles in actin-based processes [2]. WASP-deficient macrophages and monocytes have been shown to develop defects in polarization and migration in response to inflammatory chemokines *in vitro* [3,4], as well as poor formation of the actin-rich phagocytic cup [5].

WASP is composed of several functional domains, including an N-terminal enabled/vasodilator-stimulated phosphoprotein (Ena/VASP) homology 1 (EVH1) domain, also known as the WASP homology 1 (WH1) domain; a GTPase-binding domain (GBD); a proline-rich region (PRR); and a C-terminal verproline/cofilin/acidic (VCA) domain. These multiple domain structures suggest that WASP acts as an adaptor molecule, recruiting various kinds of protein tyrosine kinases, adaptor molecules, and actin-binding proteins, and connecting tyrosine kinase signaling to cellular motility devices resulting from actin polymerization [6,7].

The majority of gene mutations in WAS patients have been mapped to the WASP N-terminal region including the EVH1 domain [8], which implies that this domain is important for WASP function. To elucidate the function of the WASP N-terminal domain in the immune response, we previously developed transgenic (Tg) mice that over-express WASP exons 1–5 (aa 1–171, designated WASP15) [9]. Microglia and bone marrow-derived macrophages (BMDMs) from WASP15 Tg mice were defective in LPS-induced innate inflammatory responses [10,11]. Recently, we identified the Src homology (SH) 3 domain of Bruton's tyrosine kinase (Btk) as a binding counterpart for the WASP N-terminal domain. The WASP N-terminal domain specifically binds to the Btk SH3 domain and effectively inhibits the interaction between Btk and endogenous WASP. However, we cannot rule out the possibility that over-expression of the WASP N-terminal domain non-specifically interferes with the interaction of Btk with other PRR-containing signaling molecules, which may transduce LPS signaling.

To evaluate the significance of the specific interaction between the WASP N-terminal domain and the Btk SH3 domain in LPS signaling, we established BMDM cell lines from Tg mice expressing single-chain variable fragment (scFv) intracellular expressed antibodies (intrabodies) that specifically bind the WASP N-terminal domain. Anti-WASP scFv intrabody strongly inhibited the interaction between WASP and Btk, as well as the phosphorylation of

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WASP, resulting in down-regulation of inflammatory cytokine gene expression upon LPS stimulation in BMDMs.

2. Materials and methods

2.1. Establishment of bone marrow-derived macrophage (BMDM) cell lines

BMDMs were isolated from the bone marrow of anti-WASP scFv Tg mice [12] and cultured according as described previously [13]. The procedure for immortalizing BMDM was also described previously [11].

2.2. Immunocytochemistry

The procedure for immunocytochemistry was described previously [11]. Primary antibodies (Abs) against CD11b, F4/80, and control rat IgG (Serotec, Oxford, UK) were used. The secondary antibody was HRP-conjugated anti-rat IgG. Incubation was followed by a colorimetric substrate, 3,3′-diaminobenzidine tetrahydrochloride (DAB) (EnVision™ kits/HRP (DAB), Dakocytomation).

2.3. FACS analysis

BMDMs (5×10^5 cells) were incubated with 10 μg/mL Fc-block (anti-CD16/32 monoclonal Ab (mAb); BD Pharmingen, San Diego, CA, USA) for 10 min at 4 °C and then stained with PE-conjugated anti-CD11b (BioLegend, San Diego, CA, USA), anti-F4/80 (Serotec), anti-TLR4 Ab (BD Pharmingen), or the isotype control Ab (Immunotech, Marseille, France) for 60 min at 4 °C. After washing with PBS, cells were analyzed by flow cytometry (Bechman Coulter, EPICS XL).

2.4. Immunoprecipitation

BMDMs were lysed with RIPA buffer (Nacalai Tesque, Kyoto, Japan) for 1 h at 4 °C. The lysates were centrifuged at 10,000*g* for 10 min at 4 °C and incubated with Precleaning Matrix C (Santa Cruz Biotechnology, CA, USA) for 1 h at 4 °C to remove non-specifically bound proteins. The cleared lysates were incubated with anti-WASP (Santa Cruz Biotechnology) or anti-Btk mAb (Santa Cruz Biotechnology) and pulled down with Exacta Cruz C IP-matrix beads (Santa Cruz Biotechnology). After washing five times with PBS, immunocomplexes were re-suspended in SDS sample buffer and boiled. The immunocomplexes were immunoblotted with anti-Btk (Santa Cruz Biotechnology), anti-Myc tag pAb (MBL), anti-WASP polyclonal Ab (pAb) raised against a synthetic peptide representing WASP residues 224–238 (Upstate, Lake Placid, NY, USA), or anti-T7 tag (MBL, Nagoya, Japan).

2.5. GST pull-down assay

Preparation of GST-Btk-SH3 fusion proteins and the procedure for the GST pull-down assay was described elsewhere [11]. Pulled-down samples were immunoblotted with either anti-WASP mAb, which recognizes the WASP N-terminal domain [12], or anti-GST pAb (MBL).

2.6. Quantitative real-time PCR

BMDMs were cultured in either the presence or absence of LPS (5 μg/mL; ultra pure *Escherichia coli* 0111: B4 LPS, InvivoGen, San Diego, CA, USA) for 5 h at 37 °C. BMDMs were lysed using the Real-Time Ready Cell Lysis Kit (Roche Diagnostics, Basel, Switzerland). cDNA was obtained using the Transcriptor Universal cDNA Master (Roche) according to the manufacturer's instructions. The primer sequences and procedure for real-time PCR are described in Supplementary Table S1.

2.7. Western blot analysis of NF-κB activation

BMDMs were activated with LPS (5 μg/ml) for different time intervals at 37 °C. The activated cells were washed with PBS and lysed with SDS sample buffer at 25 °C. The cell lysates were separated by 12.5% SDS–PAGE and transferred to a polyvinylidene difluoride membrane (Bio-Rad, Hercules, CA, USA). The membrane was blocked with Blocking One (Nacalai Tesque) and probed with anti-phospho-NF-κB p65 (Ser-536), or anti-NF-κB p65 (Cell Signaling Technology, Danvers, MA, USA), followed by HRP-conjugated anti-rabbit IgG (Dakocytomation). Immunoreactive proteins were detected using Chemi-Lumi One L (Nacalai Tesque).

2.8. Tyrosine phosphorylation of WASP, Btk, and Toll/IL-1 receptor domain-containing adapter protein (TIRAP)

At 15-min after LPS stimulation, BMDMs were lysed with SDS sample buffer and immunoblotted with anti-phospho-WASP (Abcam, Cambridge, UK) or anti-phospho Btk Ab (Cell Signaling Technology). In addition, LPS-activated BMDMs were lysed with RIPA buffer (Nacalai Tesque) containing Phosphatase Inhibitor Cocktail Set (Calbiochem, Darmstadt, Germany) and immunoprecipitated with agarose-conjugated anti-phosphotyrosine (p-Tyr) mAb (Santa Cruz Biotechnology). The immunocomplexes were immunoblotted with anti-TIRAP pAb (Abcam, Cambridge, UK).

3. Results

3.1. Establishment of BMDM cell lines from anti-WASP scFv Tg mice

BMDMs prepared from anti-WASP scFv Tg mice with a C57BL/6 background were infected with a c-*myc*-containing retroviral vector, and representative BMDM clonal cell lines were established. Wild-type and WASP15 Tg BMDMs established from a wild-type C57BL/6 strain and WASP15 Tg mice with a C57BL/6 background were used for comparison. Wild-type, WASP15 Tg, and anti-WASP scFv Tg BMDM cell lines were strongly immunostained by rat monoclonal antibodies against mouse macrophages, including CD11b and F4/80 (Fig. 1A). Morphological and immunohistochemical observations suggest that these immortalized cell lines were derived from BMDMs.

Western blot analysis showed that the truncated WASP (WASP15) was strongly expressed only in WASP15 Tg BMDMs, whereas endogenous WASP was expressed at similar levels in all BMDMs (Fig. 1B, upper panel). Myc-tagged anti-WASP scFv intrabody was expressed strongly in anti-WASP scFv Tg BMDMs (Fig. 1B, lower panel). A few non-specific bands were detected, possibly due to cross-reactivity of the secondary antibody (Fig. 1B, upper panel).

3.2. Expression of TLR4 in BMDMs

TLR4, a receptor for LPS [14], is highly expressed in macrophages and transduces inflammatory signaling, such as the production of inflammatory cytokines [15]. To compare the levels of TLR4 expression between wild-type, WASP15 Tg, and anti-WASP scFv Tg BMDMs, we performed a FACS analysis with anti-TLR4 antibody. No significant difference was found in the expression of TLR4 between wild-type, WASP15 Tg, and anti-WASP scFv Tg BMDMs, and CD11b and F4/80 were also expressed at similar levels (Fig. 1C).

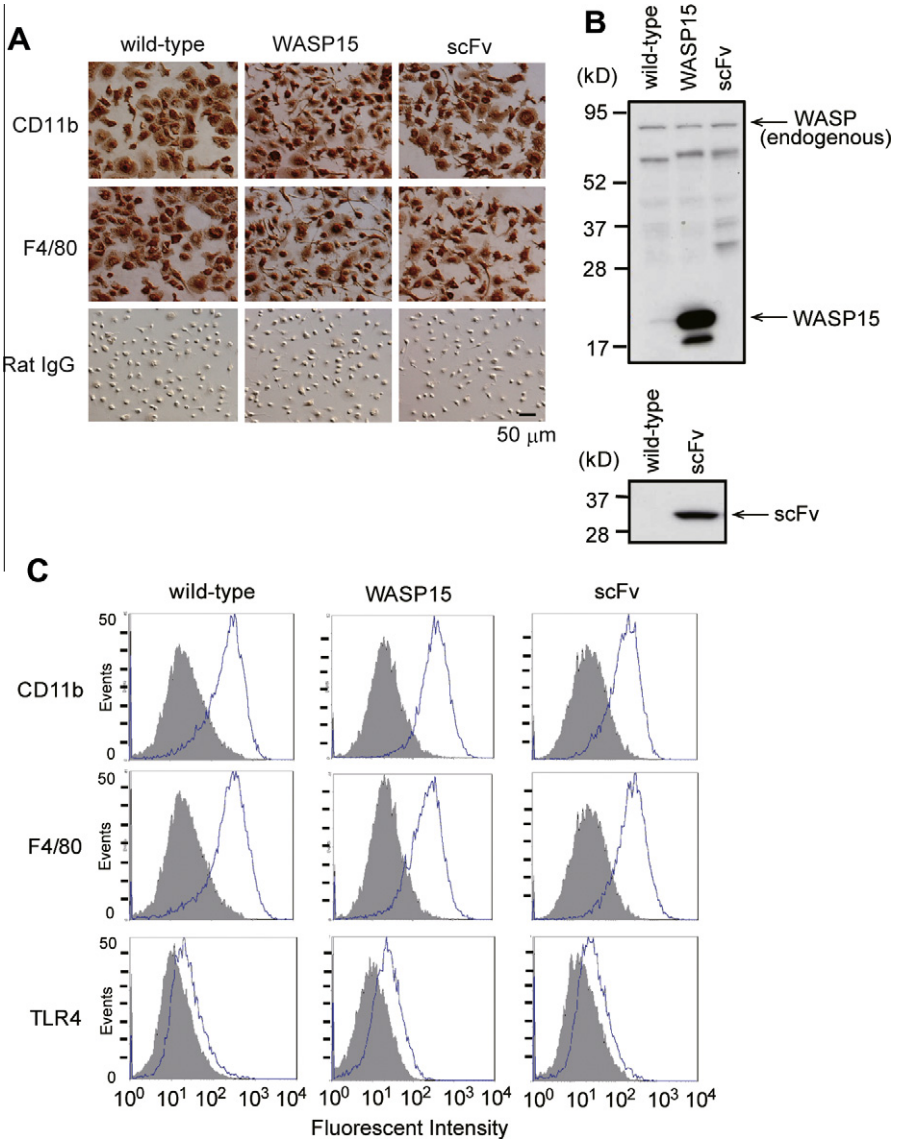

Fig. 1. Establishment of bone marrow-derived macrophage (BMDM) cell lines from anti-WASP scFv Tg mice. BMDMs were prepared from primary cultures of bone marrow and immortalized with a human c-*myc*-containing retroviral vector. (A) BMDM cell lines were immunocytochemically stained with anti-CD11b and anti-F4/80 antibodies, but not with control Rat IgG. Scale bar = 50 μm. (B) Expression of truncated WASP, endogenous WASP, and anti-WASP scFv intrabody in BMDMs. Cell lysates were analyzed by Western blotting with an anti-WASP mAb or anti-Myc tag pAb. In the upper panel, a few non-specific bands were detected due to cross-reactivity of the secondary antibody. (C) FACS analysis of wild-type, WASP15 Tg, and anti-WASP scFv Tg BMDM cell lines. Cells were stained with PE-conjugated anti-CD11b, anti-F4/80, and anti-TLR4 antibodies (open histogram), or isotype-matched control Ab (filled histogram). All results are representative of three independent experiments.

3.3. Inhibition of the specific interaction between the WASP N-terminal domain and SH3 domain of Btk by the expression of anti-WASP scFv intrabody

To assess whether anti-WASP scFv intrabody inhibits the interaction between WASP and Btk, an *in vitro* binding assay was performed using the GST or GST-Btk-SH3 fusion proteins. In contrast to the strong binding of WASP to GST-Btk-SH3 in wild-type BMDMs, their interactions were remarkably impaired in WASP15 Tg and anti-WASP scFv Tg BMDMs (Fig. 2A, upper panel). WASP15 effectively bound GST-Btk-SH3 in WASP15 Tg BMDMs (Fig. 2A, upper panel) and competitively inhibited the binding of endogenous WASP to Btk fusion protein. The levels of GST or GST-Btk-SH3 protein were similar (Fig. 2A, center panel), and the equal amount of WASP in each sample used for the pull-down assay was confirmed (Fig. 2A, lower panel). These results suggest that anti-WASP scFv intrabody specifically interferes with the specific binding between the WASP N-terminal domain and the SH3 domain of Btk, as efficiently as the overexpression of truncated WASP15.

To confirm the inhibitory effect of anti-WASP scFv intrabody on the interaction between endogenous WASP and Btk, wild-type, WASP15 Tg, and anti-WASP scFv Tg BMDM cell lysates were immunoprecipitated using anti-WASP mAb, and the immunocomplexes were immunoblotted with anti-Btk pAb. Strong binding was observed between endogenous WASP and Btk in wild-type BMDMs (Fig. 2B, upper panel). The interaction between endogenous WASP and Btk was diminished in WASP15 Tg and anti-WASP scFv Tg BMDMs (Fig. 2B, upper panel). Anti-WASP scFv intrabody strongly bound to endogenous WASP and effectively inhibited the binding of endogenous WASP and Btk, probably through intrabody masking the Btk-binding site in the WASP N-terminal domain, as shown by immunoblotting with anti-Myc tag pAb detecting Myc-tagged anti-WASP scFv intrabody (Fig. 2B, center panel).

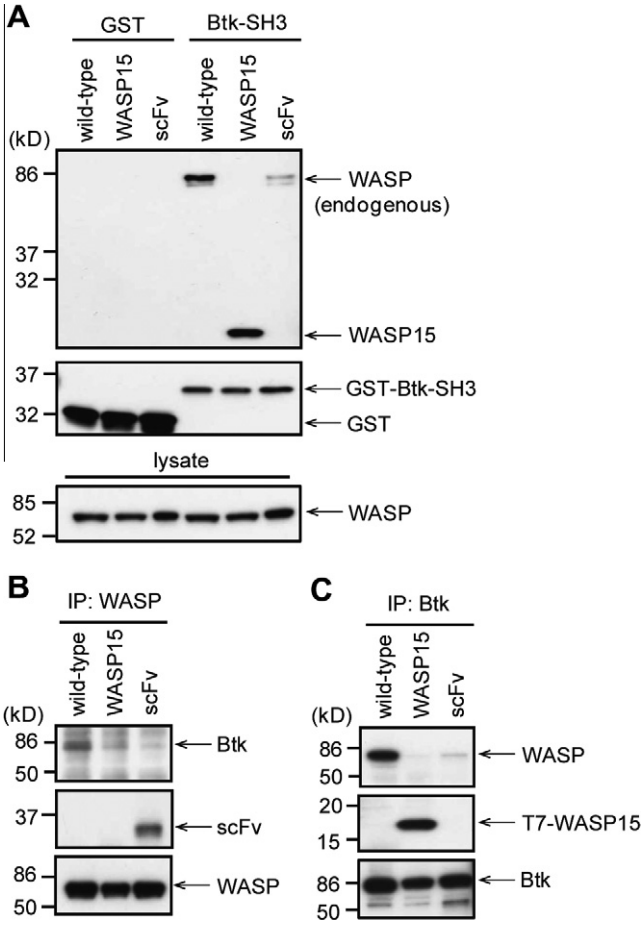

Fig. 2. Inhibition of the WASP-Btk interaction by anti-WASP scFv intrabody. (A) Wild-type, WASP15 Tg, and anti-WASP scFv Tg BMDMs were lysed and incubated with GST or GST-Btk-SH3 fusion protein non-covalently bound to glutathione Sepharose beads. Bound proteins were analyzed by Western blotting with anti-WASP mAb or anti-GST pAb. Cell lysates were analyzed by Western blotting with anti-WASP pAb. (B and C) Wild-type, WASP15 Tg, and anti-WASP scFv Tg BMDMs were lysed and immunoprecipitated with (B) anti-WASP mAb, or (C) anti-Btk mAb. Immunocomplexes were analyzed by Western blotting with anti-Btk pAb, anti-Myc-tag pAb, anti-WASP pAb, or anti-T7 tag pAb. The immunoblots are representative of three independent experiments.

Furthermore, in the reciprocal immunoprecipitation analysis using anti-Btk mAb, the specific interaction between endogenous WASP and Btk was clearly detected in wild-type BMDMs (Fig. 2C, upper panel), but their interaction was diminished in WASP15 Tg and anti-WASP scFv Tg (Fig. 2C, upper panel). Over-expressed WASP15 competitively bound to Btk and effectively inhibited the binding of endogenous WASP and Btk, as shown by immunoblotting with anti-T7 tag pAb detecting T7-tagged WASP15 (Fig. 2C, center panel). WASP and Btk was immunoprecipitated at similar levels in all BMDMs (Fig. 2B and C, lower panels). These results demonstrate that anti-WASP scFv intrabody can interfere with the interaction between the WASP N-terminal domain and Btk as efficiently as the dominant negative WASP15.

3.4. Impairment of cytokine production in anti-WASP scFv Tg BMDMs upon LPS stimulation

Macrophages activated by LPS secrete a variety of inflammatory cytokines [14,15]. To assess the inhibitory effects of anti-WASP scFv intrabody in the LPS signaling pathway, quantitative real-time PCR was performed on RNA isolated from LPS stimulated BMDM clones. Two BMDM clones (#1 and #2) independently isolated from wild-type, WASP15 Tg, and anti-WASP scFv Tg mice were used. In contrast to the marked up-regulation of TNF-α, IL-6, and IL-1β gene transcription upon LPS stimulation in wild-type BMDMs, WASP15 Tg and anti-WASP scFv Tg BMDMs expressed one-third or half of the levels of TNF-α and IL-1β transcription and half or four-fifths of the levels of IL-6 transcription (Fig. 3A). The basal gene transcription of these cytokines was not detectable level in each BMDMs (Fig. 3A and data not shown). The inhibitory effect of anti-WASP scFv intrabody was less than that by the dominant-negative WASP15, probably due to the different mechanisms underlying the interactions between the dominant negative and intrabody. These results suggest that the expression of anti-WASP scFv intrabody significantly induces the knock down of WASP function in inflammatory cytokine production following LPS stimulation in BMDMs.

3.5. Activation of NF-κB induced by LPS stimulation in BMDMs

The activation of NF-κB is essential for inflammatory cytokine production in activated macrophages [15]. To assess whether anti-WASP scFv intrabody affects the LPS-induced NF-κB signaling pathway in macrophages, the extent of LPS-induced NF-κB phosphorylation was compared between wild-type, WASP15 Tg, and anti-WASP scFv Tg BMDMs using Western blot analysis. In wild-type BMDMs, phosphorylation of NF-κB p65 (Ser-536) was rapidly induced and maintained at high levels after 15 min of LPS stimulation. In contrast, phosphorylation of NF-κB p65 was maintained at low levels in WASP15 Tg and anti-WASP scFv Tg BMDMs (Fig. 3B). Total NF-κB p65 protein levels were comparable between wild-type, WASP15 Tg, and anti-WASP scFv Tg BMDMs (Fig. 3B). However, the phosphorylation profiles of MAPKs, such as JNK, Erk1/2, and p38 MAPK, upon LPS stimulation were similar between wild-type, WASP15 Tg, and anti-WASP scFv Tg BMDMs (data not shown), suggesting that anti-WASP scFv intrabody specifically interferes with the activation of NF-κB, but not MAPKs, upon LPS stimulation in BMDMs.

These findings suggest that WASP15 over-expression and anti-WASP scFv Tg specifically block the phosphorylation of NF-κB in the LPS signaling cascade in macrophages.

3.6. Impairment of LPS-induced tyrosine phosphorylation of WASP and TIRAP in anti-WASP scFv Tg BMDMs

To assess whether anti-WASP scFv intrabody inhibits LPS-induced tyrosine phosphorylation in WASP, the extent of LPS-induced tyrosine phosphorylation was compared between wild-type, WASP15 Tg, and anti-WASP scFv Tg BMDMs using Western blot analysis. Tyrosine phosphorylation in WASP was clearly detected in wild-type BMDMs but markedly reduced in anti-WASP scFv Tg BMDMs, and almost diminished in WASP15 Tg BMDMs (Fig. 4A, upper panel). In contrast, similar levels of Btk tyrosine phosphorylation were seen in these BMDMs upon LPS stimulation (Fig. 4A, center panel). WASP and Btk were expressed at similar levels in all BMDMs (Fig. 4B, upper and center panel). These results suggest that the over-expression of the WASP N-terminal domain or anti-WASP scFv intrabody inhibits WASP tyrosine phosphorylation by interfering with Btk binding to the WASP N-terminus, but does not affect the activation of Btk upon LPS stimulation.

Toll/IL-1 receptor domain-containing adapter protein (TIRAP), also known as MyD88 adapter-like protein (Mal), acts as a bridging adaptor for TLR4 and MyD88 [16,17]. TIRAP is tyrosine phosphorylated by Btk following the activation of TLR4, ultimately leading to NF-κB activation [16,18]. To assess whether anti-WASP scFv intrabody affects LPS-induced tyrosine phosphorylation in TIRAP, the extent of TIRAP tyrosine phosphorylation was compared between wild-type, WASP15 Tg, and anti-WASP scFv Tg BMDMs.

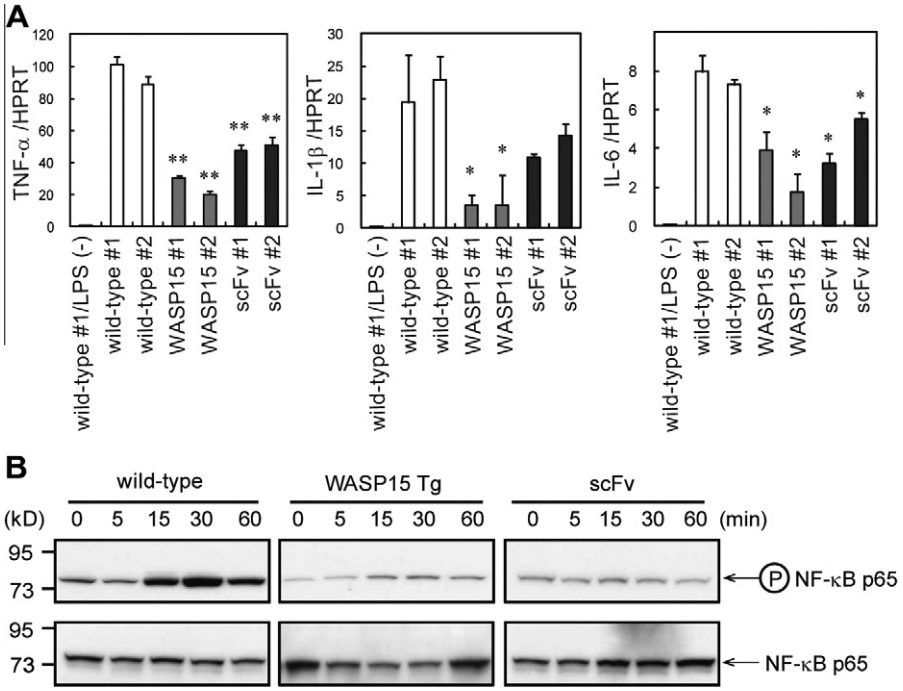

Fig. 3. Cytokine expression and phosphorylation of NF-κB induced by LPS stimulation in BMDMs. (A) Quantitative real-time PCR was performed using RNA derived from wild-type, WASP15 Tg, and anti-WASP scFv Tg BMDMs following LPS stimulation. Expression levels are reported relative to control HPRT. Clones #1 and #2 were each independently isolated from wild-type, WASP15 Tg, and anti-WASP scFv Tg mice. Values represent means ± SEs of triplicate assays. $^{*}p < 0.01$, $^{**}p < 0.001$. (B) Wild-type (#1), WASP15 Tg (#1), and anti-WASP scFv Tg (#1) BMDMs were stimulated with LPS for the time indicated and then lysed. Proteins from cellular lysates were separated by SDS–PAGE and immunoblotted with anti-phospho-specific antibody for NF-κB. Anti-NF-κB antibody was used to show equal protein loading. The immunoblots are representative of three independent experiments.

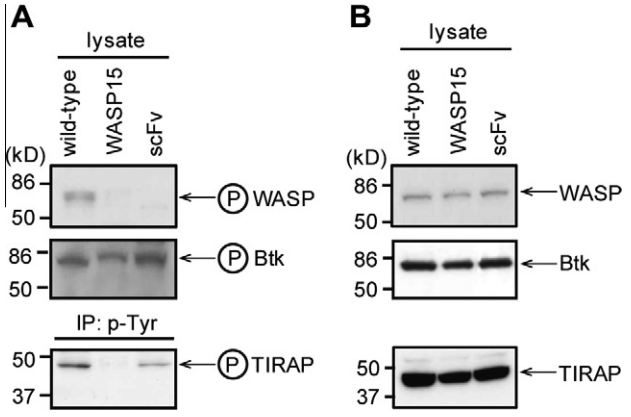

Fig. 4. Phosphorylation of WASP and TIRAP upon LPS stimulation in BMDMs. (A) LPS-stimulated wild-type (#1), WASP15 Tg (#1), and anti-WASP scFv Tg (#1) BMDMs were lysed and immunoblotted with anti-phospho-WASP pAb, anti-phospho-Btk pAb. LPS-stimulated BMDMs were lysed and immunoprecipitated with anti-phosphotyrosine (p-Tyr) mAb. Immunocomplexes were analyzed by Western blotting with anti-TIRAP pAb. The immunoblots are representative of three independent experiments. (B) Equivalent expression of WASP, Btk, and TIRAP in wild-type (#1), WASP15 Tg (#1), and anti-WASP scFv Tg (#1) BMDMs was demonstrated by Western blotting with anti-WASP mAb, anti-Btk pAb, or anti-TIRAP pAb. The immunoblots are representative of three independent experiments.

Similar to WASP, TIRAP also was sufficiently tyrosine phosphorylated upon LPS stimulation in wild-type BMDMs but not WASP15 Tg and anti-WASP scFv Tg BMDMs (Fig. 4A, lower panel), suggesting that impaired formation of the WASP-Btk complex is reflected in a reduction of LPS-induced tyrosine phosphorylation in TIRAP in WASP15 Tg and anti-WASP scFv Tg BMDMs. TIRAP was expressed at similar levels in all BMDMs (Fig. 4B, lower panel). Taken together, these observations suggest that Btk and WASP are closely associated in the complex and modulate inflammatory signals through LPS-activated TLR4 in macrophages.

4. Discussion

To extend our previous findings on the interaction between the WASP N-terminal domain and Btk in LPS-induced inflammatory responses in macrophages [11], we established BMDM cell lines from Tg mice expressing anti-WASP N-terminal domain-specific scFv intrabody. Anti-WASP scFv specifically bound the WASP N-terminal domain and interfered with the interaction of this domain with Btk, impairing inflammatory cytokine responses in BMDMs. These results imply a pivotal role of the WASP N-terminal domain and Btk in the LPS signaling cascade in macrophages.

LPS is known as a strong inducer of inflammatory cytokines in macrophages and is recognized by TLR4. Once activated, this receptor recruits adaptor molecules to form a signal complex that directs several kinase pathways and activates the transcription factor NF-κB [14,15,17]. The phosphorylation of NF-κB p65 at Ser-536 is essential for the nuclear translocation of this factor and induction of inflammatory cytokine genes [19,20]. Unlike wild-type BMDMs, phosphorylation of NF-κB p65 was greatly impaired in anti-WASP scFv BMDMs upon LPS stimulation. Thus, the WASP N-terminal domain must have critical roles upstream of the activation of NF-κB p65.

TIRAP is a membrane-associated adaptor molecule for TLR2 and TLR4 and conveys inflammatory signals to MyD88 [17]. Gray et al. demonstrated that TIRAP can be tyrosine phosphorylated by Btk following the activation of TLR2 and TLR4 [18]. TIRAP phosphorylation by Btk is required for the signaling pathway, resulting in transactivation by the NF-κB p65 subunit [21,22]. In the present study, tyrosine phosphorylation in TIRAP was clearly detected in LPS-activated wild-type BMDMs but very weakly in anti-WASP

scFv Tg BMDMs, and almost not at all in WASP15 Tg BMDMs. These results suggest that the specific interaction between WASP and Btk is important for the phosphorylation of TIRAP tyrosines to convey the LPS signal in macrophages.

Btk, a non-receptor tyrosine kinase, has been shown to play important roles in LPS-TLR4 signaling in macrophages [18,21,23]. Btk induces the phosphorylation of NF-κB p65 upon LPS stimulation and triggers inflammatory cytokine responses [21]. As we demonstrated previously [11] and in this study, inhibition of the WASP N-terminal domain and Btk impairs the phosphorylation of tyrosine residues in WASP (Fig. 4A), but Btk phosphorylation remains intact. Thus, the interaction between the WASP N-terminal domain and Btk and the resulting phosphorylation of tyrosines in WASP are important in conveying inflammatory signals upon LPS stimulation in macrophages. The phosphorylated tyrosine residue and C-terminal PRR of WASP may be targeted by other SH2- or SH3-containing molecules, which are involved in LPS signal transduction. The identification of molecules downstream of the WASP-Btk complex in the LPS signaling cascade will provide insight into the molecular mechanism underlying the inflammatory responses in macrophages.

The over-expression of dominant-negative WASP15 or scFv intrabody inhibited the interaction between endogenous WASP and Btk, impairing the inflammatory cytokine responses upon LPS stimulation. However, some differences were observed in the inhibitory effect of scFv intrabody and dominant-negative WASP15, suggesting different mechanisms of action with the two intervention strategies. In the case of dominant-negative WASP15, WASP15 binds to the SH3 domain of Btk and may broadly interfere with the interaction of Btk with other counterparts that play roles in LPS signaling. In contrast, scFv intrabody specifically binds to the WASP N-terminal domain but does not affect the function of the SH3 domain of Btk.

In conclusion, we confirmed that anti-WASP scFv intrabody strongly inhibits LPS-induced inflammatory responses by masking the Btk binding site in the WASP N-terminal region. Therefore, designing drugs that mimic anti-WASP scFv intrabody may result in new anti-inflammatory agents with fewer side effects.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2012.05.105.

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